The Dynamics of Aqueous Humor Outflow—A Major Review

Syed Shoeb Ahmad, MS,[1] Shuaibah Abdul Ghani, MS,[2] Daljit Singh MS, DSc[3] and Lott Pooi Wah, MBBS[4]

1. Specialist Ophthalmologist; 2. Consultant Ophthalmologist; 4. Resident, Department of Ophthalmology, Queen Elizabeth Hospital, Kota Kinabalu, Malaysia; 3. Consultant, Dr Daljit Singh Eye Hospital, Amritsar, India

Abstract

Aqueous humor outflow occurs through the conventional and unconventional pathway. With aging, the latter becomes less active so that the conventional pathway remains the primary mechanism of aqueous humor outflow. An abnormality of this pathway contributes significantly to disordered aqueous humor dynamics and consequent rise in intraocular pressure seen in primary open angle glaucoma and ocular hypertension. Recently, the ocular lymphatics have been implicated in aqueous humor outflow. Additionally, the trabecular meshwork is now understood to be a complex organization of structures, which are controlled by various biomechanical and biochemical mechanisms. Among others, these include the actinomyosin cytoskeletal system, extracellular matrix, intracellular signaling responses mediated by protein kinase C, Rho/Rho kinase, and other biologic factors. This review shall describe the various pathophysiologic mechanisms involved in aqueous humor dynamics.

Keywords

Aqueous humor, trabecular meshwork, glaucoma, open angle

Disclosure: Syed Shoeb Ahmad, MS, Shuaibah Abdul Ghani, MS, Daljit Singh MS, DSc, and Lott Pooi Wah, MBBS, have no conflicts of interest to declare. No funding was received in the publication of this article.

Received: March 25, 2014 **Accepted**: June 23, 2014 **Citation**: *US Ophthalmic Review,* 2014;7(2):137–42 *DOI: 10.17925/USOR.2014.07.02.137*

Correspondence: Syed Shoeb Ahmad, MS, Ophthalmology Department, Queen Elizabeth Hospital, Kota Kinabalu, 88586, Malaysia. E: syedshoebahmad@yahoo.com

A number of risk factors are associated with the causation of glaucoma. Among them, intra-ocular pressure (IOP) is an established risk factor and also one of the few factors that can be modulated to control glaucoma. The maintenance of IOP in a steady state is largely a function of aqueous humor (AH) dynamics. This is dependent on a delicate balance between AH production (inflow) and the rate of AH egress (outflow) from the eye.[1,2]

Various theories that attempt to explain the causation of glaucoma. Recently, this condition has also been suggested as a disorder of AH dynamics.[3] There are a number of biomechanical and biochemical changes occurring in the trabecular meshwork (TM), which control AH dynamics. Unlike previously when the TM was considered a passive filter, we now know that this structure is an active and complex organization of component tissues that maintain IOP in a steady state. Newer models suggest the activity of actinomyosin cytoskeletal changes, extracellular matrix organization, intracellular signaling responses mediated by protein kinase C, Rho/Rho kinase, and other biologic factors in these processes. The activity of several molecules like transforming growth factor beta2 (TGFβ2), vascular endothelial growth factor (VEGF), plasminogen activator inhibitor (PAI), endothelins, and glycosaminoglycans has also been elaborated in the literature recently to widen our understanding of the pathophysiology of glaucoma.

Taking into account these new developments, a new class of drugs is being investigated to modulate AH outflow through the conventional pathway. These experimental drugs include the latrunculins, cytochalasins, Rho/Rho-associated coiled coil-forming protein kinase (ROCK) inhibitors, and others.

This review provides a concise account of our current understanding regarding the pathophysiologic mechanisms, which modulate AH dynamics, and how an understanding of these processes is guiding us in the development of newer modalities to manage glaucoma.

Aqueous Outflow Pathways

AH is produced by the nonpigmented epithelium of the ciliary body. It flows into the posterior chamber and through the pupil, enters the anterior chamber (AC).[4–8] Aqueous outflow through the AC occurs through the following probable routes:

1. The 'conventional pathway' through the TM and Schlemm's canal (SC).
2. The 'unconventional pathway' through the ciliary muscle and other downstream tissues.
3. Through the iris surface and capillaries.

Unconventional Pathway

The uveoscleral pathway is regarded as a minor route for aqueous outflow and shall be discussed here first. Studies however show that aqueous outflow through the unconventional route can vary from 4 to 60 %.[4] The outflow rate through this route tends to decrease with age so that the

conventional pathway has to take up more function of aqueous outflow. The outflow through this route is also reduced in exfoliation syndrome, ocular hypertension, and during night-time. The outflow is found to increase in conditions such as iridocyclitis, glaucomatocyclitic crisis, and by prostaglandin analogs, which are being used to treat glaucoma successfully.

Unlike the TM/SC, the unconventional/uveoscleral pathway is not a well-defined structural pathway. In this route, AH enters the ciliary muscle and exits through the supraciliary space. It may also cross the anterior or posterior sclera and subsequently pass through the emissarial canals around the vortex veins or into the choroidal vessels. The uveoscleral outflow is driven by the pressure gradients through the uvea, movements of the ciliary muscles and changes in the extracellular matrix (ECM) or in the cytoskeleton.

The conventional route is the major site of aqueous outflow and the resistance produced in this area is responsible for the changes occurring in primary open angle glaucoma (POAG).[8]

Regions of Trabecular Meshwork

Based on anatomical location, the trabecular area can be divided into separate regions, which differ in both structure and function. These regions consist of:

1. The inner uveal meshwork.
2. The middle corneoscleral meshwork.
3. The juxtacanalicular connective tissue (JCT) adjacent to the SC.

The uveal meshwork is an irregular, net-like structure with cords connecting its different layers. There are large spaces between the cords, which contribute little to outflow resistance. This part of the meshwork consists of bands of connective tissue with irregular openings measuring 25–75 µ. The corneoscleral meshwork extends approximately 100 µ deeper. It is composed of a number of porous sheets, extending from the scleral spur posteriorly to the peripheral cornea anteriorly. The size of the openings in these sheets decrease progressively as the deeper aspects of the meshwork is reached. These openings are oval shaped and have a greater diameter of 10 µ, with a lesser axis of 5 µ. Near the SC, the lesser axis is reduced to 1–2 µ, making the mesh tighter in this region.[8–11]

The uveal and corneoscleral TM is organized into a network of trabecular beams or lamellae. Each lamella has a core, filled with a fibrillar extracellular matrix and covered by endothelial-like flat trabecular cells. The ECM is made up of an intricate arrangement of type IV collagen, versican, ADAMTS4 (a disintegrin and metalloproteinase with thrombospondin motifs-4), laminin, fibronectin, metalloproteins (MMP-2 and 14), glycosaminoglycans (GAGs), and matricellular proteins. The matricellular proteins (e.g. thrombospondins, secreted protein acidic, and rich in cysteine [SPARC], tenascin C, osteopontin, and hevin) are nonstructural adaptor proteins, which modulate the interactions between the trabecular cells and the ECM and modulate tissue remodeling.[12–16]

Unlike the uveal and corneoscleral meshworks, the JCT is not arranged into beams/lamellae, but is rather composed of a loosely arranged ECM in which a sparse number of cells are embedded. Histologically, the JCT can be divided into three layers:

1. Trabecular endothelial layer: this is continuous with the endothelium of the corneoscleral meshwork.
2. Central connective tissue layer: this consists of parallel, spindle-shaped cells loosely arranged in a connective tissue ground substance having type III collagen. Connective tissue cells also contain coated pits and coated vesicles in the plasma membrane, which are involved in receptor-mediated endocytosis.
3. Inner wall (IW) endothelium of SC: this forms the outermost part of JCT. It is a confluent layer of elongated cells attached to one another by tight junctions and lying upon a discontinuous basement membrane. It has a bumpy surface due to protruding nuclei, cyst-like vacuoles, and finger-like projections, which protrude into the lumen of SC. The IW endothelium of the SC, its basement membrane, and the adjacent JCT is known as the 'IW region'.

The JCT has a network of elastic fibers that run tangential to the IW endothelium, which is also known as the 'cribriform plexus'. In response to fluctuations in IOP, the JCT undergoes an expansion and recoil, which is an integral part of AH dynamics. Elastic fibers are known to contribute to this mechanism. An acute rise in IOP, as in rubbing of the eyes, is offset by changes in the JCT, which brings the IOP back to normal. Histologic examination of the elastic fibers reveals an inner core of cross-linked elastin with an outer sheath of microfibrillar components. There are other proteins associated with elastic fibers including myocilin, fibronectin, vitronectin, versican, tenascin C, decorin, GAG chains, laminin, fibrillin-1, microfibril-associated glycoprotein-1 (MAGP-1), and types III and VI collagen.[17–19]

Giant Vacuoles and Pores

The IW cells contain unique structures known as 'giant vacuoles'. These giant vacuoles range from 1-10 µ in width, 1-7 µ in height, and 20 µ in length. These are not intracellular structures but are out-pouchings of the endothelium caused by the pressure drop across the IW endothelium. The walls of these invaginations are very thin and in the region where the wall is most thin, unique pores are seen to form. Whether giant vacuoles serve as conduits for aqueous entry into the canal in conjunction with pores or function as a mechanism to sense pressure by stretching and allow greater fluid flow in the neighbouring intercellular junctions is unknown. In humans, reduced formation of giant vacuoles in the IW endothelium of the SC has been proposed to account for the age-related increase in outflow resistance.

The IW of SC contains approximately 20,000 transcellular pores. These pores permit the flow of AH into the SC. The majority of these pores (about 75 %) are transcellular. Others are located at the border of neighboring cells and are paracellular. IW pores range in size from 0.1 µ to more than 3 µ with an average diameter of <1 µ. The density of pores in the IW endothelium is probably less than 1,000 pores/mm^2. Some old studies had reported 1,000–2,000 pores/mm^2, but they are now attributed to fixation artefacts.[7,14,20–23]

Schlemm's Canal and Downstream Pathways

The SC is an endothelium-cell-lined canal. It runs concentrically around the eyeball at the corneoscleral junction within the internal scleral sulcus. The SC is oval or triangular in cross-section with a greater diameter of 180–250 µ. On the posterior aspect it is related to the scleral spur, while

the IW of the canal is related to the TM. Occasionally, the SC may break up into branches which coalesce again.

The lumen of the SC may collapse to a size of few microns or less at higher IOPs, which led to speculation that this might be the cause for POAG. However, studies have shown that the collapse of the SC lumen does not produce a flow resistance high enough seen in glaucomatous eyes. It is speculated that the collapse of the canal would make the condition worse and does not in itself cause glaucoma.[5]

AH from the SC drains into the 25–30 collector channels, which join the deep scleral venous plexus. From this deep plexus AH drains via an intrascleral- and episcleral-plexus into the anterior ciliary veins. Some of the collector channels bypass the deep scleral venous plexus and pass directly through the sclera. These are called the aqueous veins of Ascher, as they contain AH instead of blood. The aqueous veins ultimately drain into the conjunctival vessels near the limbus.[5]

The SC, collector vessels, and aqueous veins are subdivided by septa. These septa are present throughout the SC, but especially so near the collector channels. They bridge the inner and outer walls of the canal. The proximity of these structures to collector channel ostia suggests that their function might be to prevent complete collapse of the canal lumen and occlusion of collector channel ostia.

The collector channels and aqueous veins are relatively large vessels, which are tens of microns in diameter and generate negligible flow resistance. However, there is a case report of high IOP after the use of a surgical trabectome, suggesting the existence of considerable flow resistance distal to the SC in human eyes. Most studies, however, confirm that these vessels are not likely responsible for the elevated flow resistance seen in glaucoma. In humans, 75 % of the resistance to AH outflow is localized in the TM and 25 % occurs beyond the SC.

Increase in IOP causes progressive deformation of SC juxtacanalicular cells and trabecular lamellae with progressive enlargement of the juxtacanalicular space. This movement causes cellular elements and ECM to become less compact and reduces the ability of the juxtacanalicular space to participate as a resistance element. With prolonged high IOP, pressure and shear-mediated signals in endothelia initiate a series of responses at the cellular, molecular, and genetic levels as well as enable adaptive changes which regulate pressure and flow.

A number of platelets/plaques have also been described in the SC especially near the openings. It is not known if these plaques block the AH outflow. However, an increased number of plaques have been reported in older individuals, patients with POAG, and those with intermittent angle closure glaucoma.[7,8,13,15,19]

In POAG, three types of plaques have been described within the SC:

1. Type I plaques are of low electron density and found predominantly underneath the endothelial lining.
2. Type II plaques show a rather high electron density.
3. Type III plaques are of low electron density but often contain dark strand-like condensations with regular periodicities of about 50–100 nm.

The Role of Lymphatics in Aqueous Humor Outflow

The anterior segment of the eye is drained by lymphatics. According to Singh, aqueous pulses from the ciliary body into the posterior chamber, with every systole of the heart beat. Subsequently, there is a pulsatile entry into the AC and the angle/TM. From the angle, AH reaches the SC. Singh has proved, by passing a thin wire and also by injecting a dye, that the periphery of the cornea has a circular sinusoidal channel, which is connected to the corneal lymphatic channels, the SC, and the conjunctival lymphatics. This channel corresponds to the lucid interval seen in arcus senilis and named the Canal of Singh (COS). With every heart beat, aqueous pulses from the AC to SC and from the SC to COS through 'aqueducts of Singh'. From the COS, AH pulses in and out of the corneal lymphatic network. This to and fro pulsatile aqueous movement produces a wave pattern on tonography. With each pulse beat some aqueous escapes into the extensive conjunctival lymphatics through numerous limbal connections and ultimately reaches the general circulation.[24–27]

It remains to be seen how lymphatics play a role in the development of glaucoma.

Physiology of Aqueous Outflow

AH outflow occurs in a non-uniform or 'segmental' manner, with flow rates being higher in the areas surrounding the collector channels. At a low IOP, the IW is flat and appears to be in close proximity to a thinned-out JCT with a few giant vacuoles, while the lumen of the SC is wide open. With an increase in IOP the mechanical forces impose significant stress leading to expansion of the JCT, increased number of giant vacuoles, and distention of the JCT and IW into the lumen of the SC. The ECM composition is also found to vary in areas of high or low outflow. This either 'affects or possibly reflects' the relative flow rates which occur in the different regions of the outflow pathways.[5,17,28]

IOP becomes elevated due to increased AH outflow resistance and appears to be associated with several morphologic and biochemical changes in the TM. Various model systems have shown that activation and inhibition of contractile activity of TM cells by actinomyosin cytoskeletal integrity, myosin II phosphorylation, and ECM organization influences the AH outflow and IOP in a reciprocal manner.[29–31]

Various intracellular signaling responses mediated by protein kinase C, Rho/Rho kinase, myosin light chain (MLC) kinase, extracellular signal regulated kinase (ERK kinase), Wnt, and calcium have also been demonstrated to modulate AH outflow and regulate IOP. There are other ion channels present in TM cells including L-type Ca^{++} channel, the inwardly rectifying K^+ (K_{ir}) 2.1 channel, and swelling-activated Cl^- channels. These channels are involved in several functions, which range from volume-regulatory responses to cell contraction, thus contributing significantly to AH dynamics.[2]

The aqueous outflowing from the eye, encounters the TM endothelial cells (TMEs) first and, subsequently, the IW endothelial cells of Schlemm's canal (SCEs). When AH flows through the TMEs, it passes in the direction in which the TMEs are progressively more resistive (i.e. apical to basal). On the contrary, the SCEs face outflow in the direction in which they are less resistive (i.e. basal to apical). The net effect is that TMEs present a

greater resistance to transendothelial fluid flow compared with SCEs. The TMEs are also found to release ligands, which flow downstream and bind to the SCEs. These regulate the permeability properties of the SCEs. A number of cytokines released by TMEs are found to induce a wide variety of effects which depend upon the location of the target tissues. Interleukin-1α, interleukin-1β, and tumor necrosis factor-α are cytokines released by the TMEs. They induce cell division and migration upon binding to TMEs located near Schwalbe's line. These cytokines also induce release of matrix metalloproteinases with an increase in fluid flow across the ECM tissues.[32–34]

The TMEs apparently sense elevated IOP by the stretching and distortion produced in them. This activates an IOP homeostatic response. When the IOP is greater than the venous pressure, the increased tension makes the trabecular beams and cords taut. This triggers the stretch receptors in TMEs, leading to release of vasoactive factors, which stimulate increased outflow through the SCEs. If the IOP is less than the venous pressure, the beams and cords become flaccid, resulting in an opposite response, which increases the resistance presented by the SCEs in order to resist the reflux of blood into the SC. Mechanical stretch can also modify ion channels of TM cells specially the high-conductance Ca^{++}-activated K^+ channel (BK_{Ca}). When the cell membranes are stretched due to an increase in IOP, there is K^+ efflux from the cell cytosol. This loss of K^+ leads to a decrease in cell resting membrane potential. This activity might affect gene expression or other cell activities.[2]

Trabecular Meshwork Biochemical Properties

The TM contains 368 proteins; of which 52 are present only in glaucomatous TM. Several molecules (TGFβ2, VEGF, endothelin, PAI, and soluble CD44) are elevated in the AH of POAG patients. These molecules might play a role in influencing trabecular cells to change their 'usual' phenotype. TGFβ2 is responsible for abnormal accumulation of ECM within the TM. Studies also show that interaction of ECM components with different proteins may induce formation of deposits, which obstruct AH outflow through the TM. A protein named cochlin has been found exclusively in glaucomatous eyes. This protein is found to undergo multimerization when induced by shear stresses due to the high IOP seen in glaucomatous eyes.[35]

GAGs are negatively charged molecules found primarily on the surfaces of cells and in the ECM. There are five types of GAGs known: hyaluronic acid (HA), chondroitin sulfate, dermatan sulfate, heparan sulfate, and keratan sulfate. Apart from HA, the other GAGs are sulfated and are covalently attached to and synthesized on core proteins. Hence, they are also called proteoglycans. GAGs perform multiple functions, including cell matrix interactions, growth factor binding, and sequestration and maintenance of tissue structural integrity. Eyes with POAG have less hyaluronic acid and more chondroitin sulfate than do healthy eyes. The osmotic forces exerted by the GAGs may induce hydration (edema) of the TM, which can cause obstruction of the trabecular structure. Lysosomes are found to release catabolic enzymes, which depolymerize GAGs and prevent the obstruction of the TM. On the other hand, corticosteroids stabilize the lysosomal membranes and prevent the release of these enzymes. Molecules such as chondroitinase ABC, hyaluronidase, α-chymotrypsin, and cytochalasins have been found to reduce IOP by degrading GAGs in the ECM, disruption of actin assembly or preventing the polymerization of microtubules.[36–39]

Trabecular Meshwork Biomechanical Properties

All areas of the TM are found to contain thick bundles of actin in their cytoplasm. These filaments are responsible for a number of motile events within the cells as well as contributing to the cytoskeletal framework of the cell. These contractile elements also modulate AH outflow. Actin filaments are also essential for controlling cell shape, adherence to the ECM, motility, cytokinesis, and phagocytic activity. The cytoskeleton also regulates protein synthesis and provides a contractile apparatus for lifting of the trabecular sheets.[40–43]

The cytoskeletal elements can be divided into three major classes:

1. Microfilaments;
2. Microtubules; and
3. Intermediate filaments.

The TM is anchored by ciliary muscle tendons and fine elastin fibers, which connect to the endothelium of the SC. As the ciliary muscle contracts or relaxes it alters the shape of the TM, modifying the AH outflow through the TM and SC. Contraction of the ciliary muscle widens the intercellular spaces in the TM, increasing the permeability of tissues. There is a simultaneous decrease in uveoscleral outflow, thus the AH outflow gets distributed between the conventional and unconventional pathways, depending on the tone of the ciliary muscle. This hydraulic-like system also influences the cellular responses, particularly of the actin cytoskeleton.

On exposure to dexamethasone, some cells in human TM cultures (from both normal and glaucomatous eyes) develop complex polygons of actin known as cross-linked actin networks (CLANs). CLANs consist of a central hub, also known as vertisome, from which radiate at least five thin actin-filament bundles or spokes, which connect to adjacent hubs. Some recent studies have described somewhat similar structures, which are called CLAN-like (network CLAN, basket CLANs or starbursts). CLAN and CLAN-like arrangements are most common in the uveal and corneoscleral regions compared with the JCT. CLAN hub sites are rich in α-actinin. It is an actin cross-linking protein that belongs to the spectrin family. Spectrin is an actin cross-linking and molecular scaffold protein. It links the plasma membrane to the actin cytoskeleton and functions in the determination of cell shape, arrangement of transmembrane proteins, and organization of organelles. The hubs have also been reported to have syndecan-4. This is a transmembrane heparan sulfate proteoglycan, which interacts with actin and functions as a receptor in intracellular signaling. CLAN formation in freshly plated TM cells appears to be regulated by β1 and β3 integrins.[44–46]

The significance of CLANs and their mechanism of formation in the TM cells of the outflow system in health and disease are currently unknown. However, CLANs are the most stable form of microfilaments in the cell. This stability of CLANs may have an important functional role.

Under normal conditions, cells contain F-actin in two common patterns: diffuse arrangement of F-actin or the microfilaments forming tightly packed bundles called stress fibers. Another less-common pattern is a polygonal arrangement of actin filaments that form a geodesic dome-like structure. These structures are said to have intrinsic rigidity and contribute to cellular tensegrity.[45,47]

The F-actin arrangement in IW and JCT cells of the outflow system are disturbed in glaucoma and there is abundance of F-actin tangles among the stress fibers. Rho plays critical roles in signaling pathways that lead to formation of actin stress fibers and focal adhesions. Human ROCK 1 is a major downstream effector of the small GTPase RhoA. ROCK is a kinase belonging to the AGC (protein kinase-A, -G, -C) family of serine-threonine kinases. It regulates the movement and shape of cells (contractility) by acting on the cytoskeleton. There are two types of ROCK (ROCK-1 and -2). Human ROCK 1 is a major downstream effector of the small GTPase RhoA. Rat ROCKs were found to be the first effectors of Rho and induce the formation of stress fibers and focal adhesions by phosphorylating MLCs. Due to this phosphorylation, the actin binding of myosin II and, therefore, the contractility increases.[48–50]

As IOP increases, mechanical stress is put on the trabecular structures. In response to this stress, the elastin within the collagen beams and the elastic-like network of the JCT undergoes mechanical strain. The TM undergoes mechanical stretch (strain) in response to the increase in IOP (stress) and recoils back to its normal configuration when the IOP comes back to normal levels. As the IOP rises, the TM cells undergo increased mechanical stretch, transduced through the integrin-mediated attachments to the extracellular matrix. Any deformations in the TM are transmitted to the actin cytoskeletal network, which is altered by mechanical stretching (mechanotransduction). The TM cells respond by making the ECM more permeable to aqueous flow and hence, increase the outflow facility.[51]

H7 is a serine-threonine kinase inhibitor that blocks actinomysin contractility and increases AH outflow. Ethacrynic acid is another molecule that causes reversible cell-shape changes in the TM cells. It is associated with disruption of many components of the cytoskeleton including F-actin, α-actinin, vinculin, and vimentin. Other agents, such as the latrunculins and the cytochalasins, are also found to directly or indirectly disrupt F-actin, altering the cytoskeletal function of the TM cells and, thus, increasing the outflow facility. These agents may prove to be a new class of anti-glaucoma medications in the future.

The Rho/ROCK pathway plays an important role in the modulation of the cytoskeletal integrity of cells, synthesis of ECM components in the AH outflow tissues and in the permeability of SC endothelial cells. Activation of Rho/ROCK pathway leads to TM contraction. Rho and ROCKs are expressed in the cells of outflow pathway. They have been found in the cells of TM, JCT and SC. It is hypothesized that there is an increased expression of Rho/ROCK pathway in the outflow tissues in glaucomatous eyes. An abnormal accumulation of ECM (ECM hypothesis) and changes in contractile activity and cell adhesive interactions of the cells of aqueous outflow pathway (contractility hypothesis) are contributed to increased resistance to drainage of AH through the conventional pathway.[52,53]

The TM cells also exhibit a smooth-muscle-like phenotype based on their expression of various smooth muscle specific proteins including α-smooth muscle actin (α-SMA) and CPI-17 (protein kinase C-potentiated protein phosphatase-1 inhibitor protein). Numerous microfilament-based structures are also found in cells of the outflow pathway. These include focal contacts, adherens cell–cell junctions, and bundles of microfilaments.

Rho/ROCK pathway has a crucial role in IOP modulation. In general, the activation of Rho/ROCK pathway in the outflow tissue results in reduction of AH outflow, and thereby increases IOP, whereas the inhibition of Rho/ROCK pathway results in an increase of AH outflow, and thereby decreases IOP.

Extracellular Matrix Turnover

The ECM undergoes a normal turnover in nonglaucomatous eyes and conversely an increased deposition in POAG patients. It is not clearly understood which factors modulate the ECM turnover. An increased concentration of TGFβ2 in the aqueous of POAG patients points to a possible role for this molecule. TGFβ2 mediates fibrosis, which is just a pathologic increase in ECM deposition. *In vitro* studies have shown that TGFβ2 induces irreversible cross-linking of fibronectin by the action of tissue transglutaminase and reduced activity of MMPs. TGFβ2 is activated by matricellular protein thrombospondin-1 (TSP-1). The expression of TSP-1 is increased in a large number of POAG patients. Another downstream mediator of TGFβ2 is connective tissue growth factor. The action of TGFβ2 and connective tissue growth factor is strongly antagonized by bone morphogenetic protein-7 (BMP-7). This protein is expressed in the adult TM and modulates the action of TGFβ2.[54,55]

Conclusion

An important mechanism of glaucoma is raised IOP. In most instances IOP rises as a consequence of altered AH outflow dynamics. Thus, an understanding of the mechanisms of AH outflow is pertinent in order to develop methods to modulate these pathways. By targeting the AH outflow pathway, a newer management strategy is being developed. ■

1. Chen J, Runyan SA, Robinson MR, Novel ocular antihypertensive compounds in clinical trials, *Clin Ophthalmol,* 2011;5:667–77.
2. Llobet A, Gasull X, Gual A, Understanding trabecular meshwork physiology: a key to the control of intraocular pressure?, *News Physiol Sci*, 2003;18:205–9.
3. Mathebula SD, A review of pharmacological therapy for glaucoma, *S Afr Optom*, 2005;64:89–96.
4. Fautsch MP, Johnson DH, Aqueous humor outflow: what do we know? Where will it lead us? *Invest Ophthalmol Vis Sci*, 2006;47:4181–7
5. Overby DR, Stamer WD, Johnson M, The changing paradigm of outflow resistance generation: towards synergestic models of the JCT and inner wall endothelium, *Exp Eye Res,* 2009;88:656–70.
6. Kawa JE, Higginbotham EJ, Chang IL, et al., Effects of antiglaucoma medications on bovine trabecular meshwork cells in vitro, *Exp Eye Res* 1993;57:557–65.
7. Freddo TF, Gong H, Etiology of IOP elevation in primary open angle glaucoma, *Optom Glaucoma Soc EJ*, 2009;4(1).
8. Johnson M, What controls aqueous humor outflow resistance? *Exp Eye Res*, 2006;82:545–57.
9. Tawara A, Varner HH, Holyfield JG, Distribution and characterization of sulfated proteoglycans in the human trabecular tissue, *Invest Ophthalmo Vis Sci*, 1989;30:2215–31
10. Goel M, Picciani RG, Lee RK, et al., Aqueous humor dynamics: A review, *Open Ophthalmol J*, 2010;4:52–9.
11. Ueda J, Wentz-Hunter KK, Cheng L, et al., Ultrastructural localization of myocilin in human trabecular meshwork cells and tissues, *J Histochem Cytochem*, 2000;48:1321–29.
12. Sampaolesi R, Argento C, Scanning electron microscopy of the trabecular meshwork in normal and glaucomatous eyes, *Invest Ophthalmol Vis Sci*, 1997;16:302–14
13. Tamm ER, Fachshofer R, What increases outflow resistance in primary open-angle glaucoma?, *Surv Ophthalmol*, 2007;52:S101–S104.
14. Faralli JA, Schwin MK, Gonzalez JM, et al., Functional properties of fibronectin in the trabecular meshwork, *Exp Eye Res*, 2009;88:689–93.
15. Keller KE, Acott TS, The juxtacanalicular region of ocular trabecular meshwork: A tissue with a unique extracellular matrix and specialized function, *J Ocular Biol*, 2013;1:3.
16. Thomasy SM, Wood JA, Kass PH, et al., Substratum stiffness and latrunculin B regulate matrix gene and protein expression in human trabecular meshwork cells, *Invest Ophthalmol Vis Sci*, 2012;53:952–8.
17. Acott TS, Kelley MJ, Extracellular matrix in the trabecular meshwork, *Exp Eye Res*, 2008;86:543–61.
18. Tian B, Geiger B, Epstein DL, et al., Cytoskeletal involvement in the regulation of aqueous humor outflow, *Invest Ophthalmol Vis Sci*, 2000;41:619–23.
19. Rohen JW, Futa R, Lutjen-Drecoll E, The fine structure of the cribriform meshwork in normal and glaucomatous eyes as seen in tangential sections, *Invest Ophthalmol Vis Sci*, 1981;21:574–85.
20. Croft MA, Hubbard WC, Kaufman PL, Effect of ethacrynic acid on aqueous outflow dynamics in monkeys, *Invest Ophthalmol Vis Sci*, 1994;35:1167–75.
21. Bill A, Some aspects of aqueous humor drainage, *Eye*, 1993;7:14–9.
22. Gabelt BT, Gottanka J, Lutjen-Drecoll E, et al., Aqueous humor dynamics and trabecular meshwork and anterior ciliary muscle morphologic changes with age in rhesus monkeys, *Invest Ophthalmol Vis Sci*, 2003;44:2118–25.
23. Johnson M, Shapiro A, Ethier CR, et al., Modulation of outflow resistance by the pores of the inner wall endothelium, *Invest Ophthalmol Vis Sci*, 1992;33:1670–5.
24. Epstein DL, Rohen JW, Morphology of the trabecular meshwork and inner-wall endothelium after cationized ferritin perfusion in the monkey eye, *Invest Ophthalmol Vis Sci*, 1991;32:160–71.
25. Singh D, Conjunctival lymphatic system, *J Cataract Refract Surg*, 2003;29:632–3.
26. Singh D, Transciliary filtration and lymphatics of conjunctiva – A tale of discovery, *Tropical Ophthalmol*, 2002;2:9–13.
27. Singh D, Singh RSJ, Singh K, et al., The conjuctival lymphatic system, *Ann Ophthalmol*, 2003;35:99.

28. Nakao S, Hafezi-Moghadam A, Ishibashi T, Lymphatics and lymphangiogenesis in the eye, *J Ophthalmol*, 2012;2012:783163.
29. Keller KE, Bradley JM, Vranka JA, et al., Segmental versican expression in the trabecular meshwork and involvement in outflow facility, *Invest Ophthalmol Vis Sci*, 201;52:5049–57.
30. Stamer WD, Acott TS, Current understanding of conventional outflow dysfunction in glaucoma, *Curr Opin Ophthalmol*, 2012;23:135–43.
31. Lu Z, Overby DR, Scott PA, et al., The mechanism of increasing outflow facility by rho-kinase inhibition with Y-27632 in bovine eyes, *Exp Eye Res*, 2008;86:271–81.
32. Pattabiraman PP, Pecen PE, Rao PV, MRP4-mediated regulation of intracellular cAMP and cGMP levels in trabecular meshwork cells and homeostasis of intraocular pressure, *Invest Ophthalmol Vis Sci*, 2013;54:1636–49.
33. Alvarado JA, Alvarado RG, Yeh RF, et al., A new insight into the cellular regulation of aqueous outflow: How trabecular meshwork endothelial cells drive a mechanism that regulates the permeability of Schlemm's canal endothelial cells, *Br J Ophthalmol*, 2005;89:1500–5.
34. Alvarado JA, Yeh RF, Franse-Carman L, et al., Interactions between endothelia of the trabecular meshwork and of Schlemm's canal: a new insight into the regulation of aqueous outflow in the eye, *Trans Am Ophthalmol Soc*, 2005;103:148–62.
35. Alvarado JA, Betanzos A, Franse-Carman L, et al., Endothelia of Schlemm's canal and trabecular meshwork: distinct molecular, functional and anatomic features, *Am J Physiol Cell Physiol*, 2004;286:C621–34.
36. Bhattacharya SK, Focus on molecules: Cochlin, *Exp Eye Res*, 2006;82:355–6.
37. Belforte N, Sande P, de Zavalia N, et al., Effect of chondroitin sulfate on intraocular pressure in rats, *Invest Ophthalmol Vis Sci*, 2010;51:5768–75.
38. Knepper PA, Goossens W, Hvizd M, et al., Glycosaminoglycans of the human trabecular meshwork in primary open-angle glaucoma, *Invest Ophthalmol Vis Sci*, 1996;37:1360–7.
39. Pang IH, Clark AF, Drug-induced hydrolysis of extracellular matrix: a novel therapeutic mechanism for primary open angle glaucoma, *J Med Sci*, 2004;24:173–8.
40. Hubbard WC, Johnson M, Gong H, et al., Intraocular pressure and outflow facility are unchanged following acute and chronic intracameral Chondrotinase ABC and hyaluronidase in monkeys, *Exp Eye Res*, 1997;65:177–90.
41. Cai S, Liu X, Glasser A, et al., Effect of latruncilin A on morphology and actin-associated adhesions of cultured human trabecular meshwork cells, *Mol Vis*, 2000;6:132–43.
42. Epstein DL, Rowlette LL, Roberts BC, Acto-myosin drug effects and aqueous outflow function, *Invest Ophthalmol Vis Sci*, 1999;40:74–81.
43. Gipson IK, Anderson RA, Actin filaments in cells of human trabecular meshwork and Schlemm's canal, *Invest Ophthalmol Vis Sci*, 1979;18:547–61.
44. Clark AF, Wilson K, McCartney MD, et al., Glucocorticoid-induced formation of cross-linked actin networks in cultured human trabecular meshwork cells, *Invest Ophthalmol Vis Sci*, 1994;35:281–94.
45. Hoare MJ, Grierson I, Brotchie D, et al., Cross-linked actin networks (CLANs) in trabecular meshwork of the normal and glaucomatous human eye in situ, *Invest Ophthalmol Vis Sci*, 2009;50:1255–63.
46. O'Rielly S, Pollock N, Currie L, et al., Inducers of cross-linked actin networks in trabecular meshwork cells, *Invest Ophthalmol Vis Sci*, 2011;52:7316–24.
47. Wade NC, Grierson I, O'Rielly S, et al., Cross-linked actin networks (CLANs) in bovine trabecular meshwork cells, *Exp Eye Res*, 2009;89:648–59.
48. Rocha-Sousa A, Rodrigues-Araujo J, Gouveia P, et al., New therapeutic targets for intraocular pressure lowering, *ISRN Ophthalmol*, 2013;2013:261386.
49. Rao PV, Deng PF, Kumar J, et al., Modulation of aqueous humor outflow facility by the Rho Kinase-specific inhibitor Y-27632, *Invest Ophthalmol Vis Sci*, 2001;42:1029–37.
50. Sabany I, Tian B, Gabelt BT, et al., Functional and structural reversibility of H7 effects on the conventional aqueous outflow pathways in monkeys, *Exp Eye Res*, 2004;78:137–50.
51. WuDunn D, Mechanobiology of trabecular meshwork cells, *Exp Eye Res*, 2009;88:718–23.
52. Honjo M, Tanihara H, Inatani M, et al., effects of Rho-associated Protein Kinase inhibitor Y-27632 on intraocular pressure and outflow facility, *Invest Ophthalmol Vis Sci*, 2001;42:137–44.
53. Yang C-YC, Liu Y, Lu Z, et al., Effects of Y27632 on aqueous humor outflows facility with changes in hydrodynamic pattern and morphology in human eyes, *Invest Ophthalmol Vis Sci*, 2013;54:5859–70.
54. Johnson DH, The effect of cytochalasin D on outflow facility and the trabecular meshwork of the human eye in perfusion organ culture, *Invest Ophthalmol Vis Sci* 1997;38:2790–9.
55. Wordinger RJ, Fleenor DL, Hellberg PE, et al., Effects of TGF-β2, BMP-4 and gremlin in the trabecular meshwork: implications for glaucoma, *Invest Ophthalmol Vis Sci*, 2007;48:1191–200.