The Dynamics of Aqueous Humor Outflow—A Major Review
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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

A number of risk factors are associated with the causation of glaucoma. Among them, intraocular 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 actinomysin 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
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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).

**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.

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.

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, tenasin C, decorin, GAG chains, laminin, fibrillin-1, microfibril-associated glycoprotein-1 (MAGP-1), and types III and VI collagen.

**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 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/mm². Some old studies had reported 1,000–2,000 pores/mm², but they are now attributed to fixation artefacts.

**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.3

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 trabeculectomy, 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, 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

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+) 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

**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.26–27

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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.20–31

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 Ca2+-activated K+ channel (BKCa). 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 muetamization 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 in the 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.35-46

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 JC 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.4-10

As IOP increases, mechanical stress is put on the trabecular structures. In response to this stress, the elastic within the collagen beams and the elastic-like network of the JT 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.21

H7 is a serine-threonine kinase inhibitor that blocks actinomycin 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, JT 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.22,23

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 TGFβ2.24,25

**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.

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