

AQUEOUS HUMOR: PHYSIOLOGY AND DYNAMICS

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Abstract: This article reviews the anatomy and physiology of aqueous humor circulation, from secretion to drainage. There are also highlighted the secretory mechanisms of the ciliary body, the blood-aqueous barrier, the pathways of aqueous flow within the eye and the aqueous outflow system.

Aqueous humor is a transparent colourless solution that fills the anterior chamber of the eye, being produced by the nonpigmented cells of the ciliary epithelium. It has several important functions in ocular physiology: nutritive, optical and mechanical. At the beginning of the 20th century, aqueous humor was considered a stagnant fluid. This idea was revoked after several experiments designed to investigate it and other aspects of the anatomy and physiology of aqueous drainage were discovered later. Aqueous humor is secreted into the posterior chamber, passes through the pupil in the anterior chamber and drains mostly into venous circulation through the conventional (pressure dependent) pathway. This is composed of trabecular meshwork, Schlemm's canal, scleral collector channels and aqueous and episcleral veins. The remainder goes into the orbit through the uveo-scleral (non-conventional, pressure independent pathway), composed of interstices of the ciliary muscle, ciliary body lymphatics, the suprachoroidal space and the sclera.

Formation and secretion of aqueous humor

Aqueous humor is continuously formed by the cells of the non-pigmented ciliary epithelium. Three physiologic processes are involved in aqueous humor formation: diffusion, ultra filtration and active secretion. The first two are passive processes, requiring no active cellular participation. Diffusion takes place down a concentration gradient. High-lipid solubility substances can easily penetrate biological membranes in this way. The process of ultra filtration takes place because of gradients in fluid pressure between the different compartments of the eye. This process controls the flow of blood plasma across the fenestrated ciliary capillary endothelia, driven by hydrostatic pressure. It is responsible for the formation of the reservoir of plasma ultra filtrate in the stroma, from which aqueous humor is derived through active secretion. There is a limited influence of systemic blood pressure on intraocular pressure (IOP) and increased IOP reduces aqueous inflow, showing a responsiveness of aqueous inflow to altered hydrostatic pressure gradients. The mechanism of cellular secretion is better understood than that of ultra filtration.

The concentration of Na⁺, K⁺, Cl⁻, bicarbonate, glucose, some amino acids and other organic compounds in aqueous humor are maintained by specific transport systems in the ciliary epithelium. Active secretion requires energy, provided by hydrolysis of ATP. This energy is used to secrete substances against a concentration gradient. Active secretion

accounts for 80-90% of total aqueous humor formation. Through active secretion substances are moved in a direction opposite to that which would be expected by passive mechanisms alone. Aqueous humor exhibits increased lactate, ascorbate and certain amino acid concentrations as compared to plasma, as a consequence of active secretion.

In the ciliary epithelium, there have been identified several membrane active transport systems, including Na⁺ K⁺ATP-ase, carbonic anhydrase, Na⁺K⁺-2Cl⁻ symport parallel Cl⁻/HCO₃⁻ and Na⁺/H⁺ antiports as well as amino acid membrane transporters.(1) The evidence for active secretory processes in the ciliary epithelium is provided by the inhibition of aqueous humor inflow by the inhibitors of cellular enzymes. There is a reduction of IOP after experimental topical and intravitreal administration of Na⁺K⁺ATP-ase inhibitors, ouabain and vanadate. Vanadate ion reduces IOP in rabbits but does not lower IOP in ocular hypertensive patients.(2) An active transport system is characterized by a limit beyond which an increase in substrate produces no further increase in transport.

When this limit is reached the system is saturated. For instance, ascorbate transport system in the eye is saturable. Electrophysiological studies of the isolated ciliary epithelium have demonstrated the need for Na⁺ and HCO₃⁻ for the maintenance of indices of ion transport or secretion across the membranes (transepithelial potential difference and short-circuit current).(1) The anionic transport systems of the anterior uvea have a strong relationship to those of the kidney and liver. The process of aqueous humor formation is very much alike to the formation of the cerebrospinal fluid.

Aqueous humor composition

Aqueous humor has a refractive index of 1.336, lower than that of the cornea. Because of this fact, there is a slight divergence of the light rays as they pass the cornea-aqueous interface. The density and the viscosity of the aqueous humor are lower than that of water and the osmolality is slightly higher of that of plasma. The volume of human anterior chamber is approximately 200 μL, whereas that of the posterior chamber is approximately 60μL. The main difference between aqueous and plasma is found in the very low protein content of the aqueous which is in the region of 0.5% of plasma.(1)

The composition of protein in the aqueous is also different from that in plasma. There are far less high molecular weight proteins such as beta lipoproteins and immunoglobulin in the aqueous than in the plasma. IgG is present in aqueous at a

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concentration of 3mg per 100ml, whereas IgM, IgA and IgD are absent.(3) In eyes with uveitis, the level of IgG increases and IgA and IgM also appear. There also have been reported elevated levels of aqueous matrix metalloproteinase (MMP-2) and tissue inhibitors of MMP (TIMP-1, TIMP-2 and TIMP-3) in p with myopia in a stationary stage and with an axial length greater than 26 mm.(4) In aqueous humor there are also trace quantities of complement proteins and of components of fibrinolytic and coagulation systems, excepting plasminogen and plasminogen proactivator which are present at more significant levels. Only traces of the inhibitors of plasminogen activator are present, ensuring that the aqueous outflow pathway remain free of fibrin.

There are also in aqueous humor small quantities of mono- and dinucleotide that play a role in the control of corneal endothelium ion transport. α and γ -lens crystallins are also present in small quantities in healthy eyes and their levels increase in cataract. The amino acid concentration is frequently higher in the aqueous than in the plasma. It has been suggested the existence of six transport systems for amino acid in the ciliary epithelium: three independent for neutral amino acids and separate mechanisms for basic aminoacids, acidic amino acids and urea. Proteomic analysis of the human aqueous humor indentified 676 nonredundant proteins.(5) The elucidation of aqueous proteome will establish a foundation for protein function analysis and identification of differentially expressed markers associated with diseases of the anterior segment. Open angle glaucoma patients (primitive and pseudoexfoliative type) have elevated levels of multiple biomarkers of Alzheimer disease in aqueous humor, compared with cataract patients. They have elevated levels of apolipoprotein AI, Apolipoprotein CIII, transthyretin (TTR), complement factor H, and complement C₃ and α_2 macroglobulin.(6)

In aqueous humor there are very high concentrations of ascorbate and lactate. Ascorbate is actively secreted in aqueous humor and its production depends on the presence of ATP and Na⁺ gradient. Ascorbate is concentrated mainly by the lens epithelium and has a protective effect against UV-induced DNA damage to this tissue. Ascorbate has an antioxidant role, partially absorbs UV radiation and regulates the sol-gel balance of mucopolysaccharides in the trabecular meshwork. Lactate accumulates in the anterior chamber, being produced by both ciliary body and retina. In anterior chamber lactate concentration is considerably higher than in plasma.

Aqueous humor also contains hydrogen peroxide, as a result of reactions between ascorbic acid and trace metals. Human trabecular cells exposed to 1 mM hydrogen peroxide show reduced adhesiveness to the extracellular matrix proteins fibronectin, laminin and collagen types I and IV.(1) Repeated oxidative stress in vivo may result in reduced trabecular meshwork cells adhesion leading to cell loss, one of the major histopathologic changes in glaucoma. Glucose concentration in aqueous humor is slightly less than that in plasma. Glucose diffuses into the aqueous and also in the cornea. Its concentration in the corneal endothelium is half of that in aqueous.

Oxygen is also present in aqueous humor as well as transforming growth (TGF) β_2 factor that may play a role in glaucoma pathogenesis. The intrinsic activity of TGF β_2 contributes to the maintenance of the anterior chamber associated immune deviation. Many physiologic systems, including the central nervous system, endocrine and cardiovascular systems, as well as change in metabolic activity influence the secretion of aqueous humor. The aqueous production shows a diurnal cycle, decreasing by as much as 50% at night.(8) The aqueous diurnal cycle is influenced by

circulating catecholamines, epinephrine and norepinephrine, and by the activity of the subject. Aqueous production diminishes slightly with age and by now there is no report of an age-dependent loss of ciliary epithelial cells. Hypothermia leads to a decrease in aqueous humor formation, reflecting a deactivation of metabolic processes necessary to maintain active secretion.

The ultrafiltration component of aqueous formation is pressure sensitive, decreasing with increasing IOP. This phenomenon is called pseudofacility and is quantifiable. Decreased plasma osmolality reduces aqueous formation, and also does uveitis, especially iridocyclitis. There are also hormonal influences on aqueous secretion and many pharmacologic agents reduce aqueous secretion and thus IOP. They are largely used in glaucoma treatment, including β -adrenoceptor antagonists such as timolol, betaxolol and others, carbonic anhydrase inhibitors, and α_2 -adrenoceptor agonists such as brimonidine. Thicker corneas may be associated with lower aqueous production and lower uveo-scleral outflow.(9)

Blood aqueous barrier

Large molecules such as protein are present in aqueous in small quantities although their plasma concentration is high. In humans, normal plasma total protein levels are 6g per 100 mL, and in aqueous humor there are less than 20 mg protein per 100 mL (less than 0.5% of plasma concentration). This is due to the existence of blood-aqueous barrier, an epithelial barrier formed by the nonpigmented ciliary epithelium and the posterior iridial epithelium and by the endothelium of the iridial vessels. Both these compounds have tight junctions of the "leaky" type. There is some evidence indicating that the tight junctions of the ciliary epithelium have few sealing strands, these being responsible for a low transepithelial resistance characteristic of the less tight and somewhat more "leaky" epithelia.(7) Thus, the blood-aqueous barrier is not absolute. The greater the lipid solubility of a molecule, the greater its ability to penetrate the barrier and to pass into the posterior chamber. Certain substances, such as urea, creatinine and some sugars penetrate the blood-aqueous barrier but they are slower than across capillary walls. The blood-aqueous barrier is fragile and may be disrupted by various stimuli such as corneal abrasions, uveal inflammation, intraocular infections, paracentesis, intraocular surgery, topical applied drugs (anticholinesterase agents). The resultant aqueous produced is the "secondary" aqueous with a marked increase in protein concentration. Certain substances as mannitol penetrate poorly the blood-aqueous barrier and are used clinically to reduce IOP. They accumulate in the extacellular spaces of the body, producing a high osmotic pressure that draws water from cells and ocular fluids leading to a reduction of IOP.

Aqueous humor outflow

Aqueous humor produced in the ciliary body and released in the posterior chamber passes through the pupil in the anterior chamber and then through trabecular meshwork, Schlemm's canal and aqueous veins. The fluid movement is directed by pressure change. In the eye normal IOP is 15 Hg, but pressure drops to 9 mm Hg in SC and further to 7-8 mm Hg in aqueous veins.(10)

There is a mechanosensing activity in trabecular meshwork and Schlemm's canal, including ways in which cells transfer mechanical changes into biologic signals. There is no direct evidence of baroreceptor activity in the eye but some evidence for an "ocular baroreflex" is given by the fact that eye pressure is tightly regulated over the entire life and eyes exposed to stretching and increased fluid flow return to starting IOP levels. The most likely site for this baroreceptor activity in the conventional outflow pathway appears to be the interface between the juxtacanalicular region of the trabecular meshwork

and the Schlemm's canal.(10) The cells and extracellular matrix in this region are considered the site of outflow resistance in glaucoma.

The Schlemm's canal inner wall has two diametrically opposed functions. First, it must allow aqueous to pass in a basal to apical direction, facilitating entry into the canal lumen. Second, the Schlemm's canal is part of the blood aqueous barrier along with the ciliary epithelium, the iris vascular endothelium and the posterior iris epithelium.(11) This barrier formed by the tight junctions between the endothelial cells of the inner wall of the canal prevents blood products from entering the eye when elevated episcleral venous pressure exceeds IOP. The factors that regulate the blood aqueous barrier at the level of Schlemm's canal are poorly understood. Schlemm's canal endothelium stretches and expands as a response to pressure, in both size and contractile ability. Canalicular endothelial cells form more giant vacuoles and pores increased in size as a response to pressure. Schlemm's canal cells undergo shear stress much alike to that in large arteries and act by aligning in the direction of flow.

The pressure in the outflow system is influenced by pulse, blinking and head movement, indicating that fast and slow adaptation mechanisms may be present to respond to rapid pressure change. Tissue and cell stiffness are factors that may alter the responsiveness of trabecular meshwork and Schlemm's canal to fluid flow, shear stress and pressure. There are changes in cytoskeleton, morphology, protein and gene expression produced in trabecular meshwork cells as a result of changing substrate stiffness.(10) It is well known that trabecular meshwork stiffness increases in primitive open angle glaucoma with regional variability.

It has been proved that drug treatments also modify Schlemm's canal stiffness. If drug treatment increased Schlemm's canal stiffness, resistance to outflow also increased. If drug treatment relaxed the cells, resistance decreased. In Schlemm's canal cells vacuole and pore formation is pressure dependent and is influenced by stiffness. The number of pores is reduced in glaucoma tissue. Increased trabecular and canal stiffness with age or primitive open angle glaucoma could reduce baroreceptor activity and pore formation, increasing outflow resistance. Thus drugs that modify cytoskeleton directly or indirectly, such as Rho Kinase inhibitors and latrunculins, may decrease cell stiffness and reduce outflow resistance.

Defining the mechanosensing activity of the conventional outflow pathway may be the clue for new therapeutic options in primitive open angle glaucoma. Latrunculins A and B significantly reduced IOP and were consistent in their facility increasing effect in living cynomolgus monkey.(12) This indicates that active disorganization of the actin cytoskeleton in the trabecular meshwork by latrunculins may be an useful antiglaucoma strategy. Effects on corneal endothelium or ciliary epithelium (pseudoguttata, increased central corneal thickness) are a potential safety issue.

The extracellular matrix in the juxtacanalicular region of the trabecular meshwork is responsible for a large part of outflow resistance in glaucoma. It is composed of elastin, collagens, laminin, fibronectin and fibrillin and increases with age and in primitive open angle glaucoma. This increase is primarily seen in "sheath-derived plaque material" which correlates with axonal damage in glaucoma. Matrix metalloproteinases, tissue inhibitors of matrix metalloproteinases and other inhibitors of these substances are involved in remodeling and maintaining of extracellular matrix. Another important factor in modulating outflow resistance is the presence of various glycosaminoglycans that are present in the intertrabecular spaces. Changes in the extracellular matrix bring

the cells in contact with integrins that activate integrin-signaling. This outside-in signalling affect outflow facility by regulation of cell contractility. In addition, inside-out signaling can be initiated by growth factor receptor pathways or by G-protein-coupled receptors that induce activation of the integrin. Eleven integrins are expressed by the TM cells.(10)

In addition, an uveo-scleral outflow pathway exists, that accounts for nearly half of total aqueous drainage in young human eyes. New evidence suggest ocular lymphatics, formerly believed to be absent in the eye, may represent an uveolymphatic exit route for fluid and proteins retained in the uveo-scleral tissue. A minimal amount of fluid leaves the anterior and posterior chambers through the iris and through the vitreous to the optic nerve and retinal vessels.(1)

CONCLUSIONS

Aqueous humor is a liquid of paramount importance in the homeostasis of normal human eye. Apart of maintaining the IOP and the internal alignment of intraocular structures, it nourishes the ocular structures that are necessarily avascular, the posterior cornea, the crystalline lens and the anterior vitreous. During ocular diseases treatment very effort must be made for its normal production and existence inside the eye.

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