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Aqueous humor's biochemical composition in ocular pathologies

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Abstract

Background: Being analogous to a blood surrogate, the aqueous humor has an important role in the regulation of the homeostasis of the ocular tissues. It has many functions: provides nutrition, removes excretory products, transports neurotransmitters, stabilizes the ocular structures, influences the intraocular pressure, and participates in the immune response against invading pathogens and inflammation. Aqueous humor's unique composition (electrolytes, proteins, biologically active substances, and organic solutes) is required to maintain adequate functionality of the ocular system. Its secretion is a complex biochemical reaction that gives specific properties and makes the difference from other human fluids. Different factors (traumatic, physical, chemical, pharmacological) and eye pathologies influence its composition, modifying its physiological properties, and cause pathological conditions in the anterior segment. In the last decade, it was made massive progress in the characterization of the composition of aqueous humor in different pathologies (glaucoma, myopia, keratoconus, age-related macular degeneration, branch retinal vein occlusion, etc.). It determined the biomarkers for eye's pathologies and identified the progression of the disease.

Conclusions: The detailed knowledge of biochemical and physiological properties of aqueous humor is necessary in understanding the pathophysiology of eye's diseases. The significant variations in the differentially abundant changes in human aqueous humor may be relevant for future diseases treatment in order to get favorable outcomes in patients. Specific markers for pathologies represent nowadays an important field of research. These markers are necessary for early diagnosis and selecting the proper treatment for each individual case by stopping the clinical disease progression.

Keywords: aqueous humor, composition, pathological conditions.

Introduction

Aqueous humor (AH) is the biological fluid produced by the ciliary body in the posterior chamber and fills both chambers (anterior and posterior) [1]. It supplies nutrients and oxygen and removes metabolic waste and toxic substances from posterior cornea, lens and maybe the anterior vitreous [2]. AH provides an optically clear medium for vision, maintains intraocular pressure (IOP) and structural integrity of globe, it has a protective role against ultraviolet [3] and facilitates cellular and humoral responses of the eye to inflammation and infection [4, 5]. Aqueous humor also permits drugs to be distributed to different ocular structures [6].

All AH's properties are due to unique chemical composition. To reach the posterior chamber, the various constituents of aqueous humor must traverse the three tissue components of the ciliary processes – the capillary wall, stroma, and epithelial bilayer [7]. All these structures compose the blood-aqueous barrier which is responsible for the AH's properties [8].

Aqueous humor's dynamics and secretion

The ciliary body represents the main site of aqueous production, secreted into the posterior chamber, AH passes through the pupil in the anterior chamber where it leaves the eye by passive flow via two pathways – conventional and non-conventional route (both located in the iridocorneal angle of the eye). The trabecular meshwork represents the conventional pathway, it is across the inner wall of Schlemm's canal where the AH is drained into its lumen, and after this into the collector channels, aqueous veins and episcleral veins. The uvealscleral pathway refers to the leaving of AH through intercellular spaces among ciliary muscle by diffusion into the suprachoroid and out through the sclera [5, 7, 9, 10, 11].

AH is secreted by ciliary processes, each of which is composed of a double layer of epithelium over a core of stroma and rich supply of fenestrated capillaries [12]. The two layers of the epithelium (pigmented and nonpigmented cells) are with the apical surfaces in apposition to each other [13, 14]. The nonpigmented epithelium has shown to have a large number of mitochondria, rough endoplasmic reticulum, zona occludens, lateral and surface interdigitations. These cells are considered the actual site of AH production. The pigmented epithelium contains numerous melanin granules. The non-pigmented layer is in contact with the aqueous humor in the posterior chamber, and an external, pigmented layer in contact with the ciliary process stroma [7, 12, 15]. Sympathetic and parasympathetic nerves supply the ciliary body [16].

The secretion involves three main processes: diffusion, ultrafiltration and active secretion [5]. Diffusion and ultrafiltration are passive, do not require cellular participation [17, 18, 19] and are responsible for the accumulation of plasma ultrafiltrate in the stroma. Diffusion involves the

passive movement of ions, based on charge and concentration. Ultrafiltration is a pressure-dependent process-IOP, osmotic pressure of blood and in the ciliary body (the difference between the hydrostatic pressure and IOP favors fluid movement – water and water-soluble substances) [10, 11, 12]. Active secretion needs energy (provided by hydrolysis of ATP-adenosine triphosphate) and is responsible for approximately 80% to 90% of the total aqueous humor formation by the movement of ions and other molecules across a concentration gradient in blood-aqueous barrier [2, 19, 20].

AH formation is a complex process and it began with the pass of an ultrafiltrate through the fenestrated capillaries of the ciliary processes into the stroma. The ultrafiltrate contains a high percentage of proteins, which is important for filtration from the capillaries. A number of solutes are transported from the ultrafiltrate to the posterior chamber across the ciliary epithelium, meaning the extraction of electrolytes and other substances (glucose, amino acids, ascorbate, etc.) against a concentration gradient, by means of diffusion, active or carrier-mediated secretion of solutes [8, 20].

The active process of AH secretion is mediated by two enzymes, which are present in ciliary epithelium – Na^+-K^+- ATPase and carbonic anhydrase [4, 7, 21, 22]. The gap junctions have the role of conducting water in the condition of a high degree of ion coupling [5]. Solutes, primarily Na^+ and Cl^-, and water are transferred from the extracellular stroma of the ciliary processes to the posterior chamber by sequential passage through the pigmented ciliary epithelial cells (PE), gap junctions (direct communication between the two cells, layers of ciliary epithelium at the apical-apical interface), and nonpigmented ciliary epithelial (NPE) cells. Na^+ is ejected through Na^+-K^+- activated ATPase, and Cl^- is released through Cl^- channels at the basolateral surface of the NPE into the aqueous humor [5, 11, 20, 22]. In ocular tissues the enzyme has a special function: control of the corneal hydration and the production of AH [21].

Na^+-K^+- ATPase is the enzyme responsible for Na^+ and K^+ transport and has 4 subunits (2α and 2β-subunits). It generates an electrochemical gradient across the membrane [11, 23]. The enzyme has the function of maintaining the intracellular ionic balance. In the non-pigmented epithelial cells the Na^+-K^+- ATPase excludes the Na^+ at the surface of the cells, causing local accumulation of Na^+ and generates a hyperosmotic environment with the formation of AH by driving water and anions [11].

The carbonic anhydrase has the role of pH regulation, CO_2 and HCO_3^- transport and water and electrolyte balance [11]. Other channels responsible for the transport of fluid are Aquaporins (AQPs). AQPs are transmembrane water channels that contribute to AH secretion, especially AQP1 and AQP4 found on ciliary epithelium, trabecular meshwork and endothelium of the canal of Schlemm, specific for AQP1 [24, 25]. Water transport through them occurs after a local osmotic gradient is established via secretion of ions (Na^+, Cl^-, and HCO_3^-) and small molecules (ascorbic acid) [7, 26, 27]. The role of these specific AQPs in AH production has been identified as potential therapeutic targets for pharmacological inhibition in glaucoma patients [28].

All these biochemical reactions involved in AH secretion prove the liquid's unique composition and functions.

Aqueous humor's biochemical composition

AH is considered to be analogous to interstitial fluid in that no red blood cells are present in it, and it is the source of nourishment for cells of the corneal endothelium, stromal keratocytes and the entire lens [21, 29]. All the physiological properties of AH (refractive index 1.336, pH 7.3 [30, 31]) maintain proper functionality of the ocular system [32]. Human AH is presented as a complex mixture of electrolytes, organic solutes, growth factors, cytokines, proteins that provide the metabolic requirements to the avascular tissues of the anterior segment [33]. These differences make AH's viscosity and density a little higher than that of pure water, while the osmolarity is slightly higher than that of plasma [31]. Due to small eye's chambers (anterior with a volume of 200μl and posterior with a volume of 60 μl [34, 35]), it is hard to do a proper chemical analysis of AH in ocular pathologies, anyway, there were several studies that tried to focus the main differences. We will discuss the most relevant components in AH composition.

The greatest difference between human AH and plasma resides in the very low protein and high ascorbate concentration in the aqueous (tab. 1) [21, 35].

Table 1

Aqueous and Serum protein concentration [21, 35]

Chemical composition	Aqueous	Serum
Total protein	0.013g/100ml	7.5 g/100ml
Globulin	0.003 g/100ml	2.5 g/100ml
Albumin	0.010 g/100ml	5 g/100ml
Ascorbic acid	19 mg/100ml	1.3 mg/100ml
Glucose	47mg/100ml	98mg/100ml

The levels of these constitutes are thought to be involved in the development of several eye diseases [36], and investigating the AH will facilitate generation of new hypotheses regarding the etiology of such pathologies [37].

In the article, there were pointed out the most frequent ocular pathologies that cause blindness: glaucoma, uveitis, and diabetic retinopathy, and their changes in the AH's composition that can be named biomarkers. The variations in AH may be relevant for future diseases treatment. All three pathologies are a significant public health problem, being the leading cause of irreversible visual loss. Glaucoma is a group of optic neuropathies characterized by progressive degeneration of retinal ganglion cells and appears at subjects older than 40 years. It affects more than 70 million

people worldwide with approximately 10% being bilaterally blind [12, 38]. Uveitis is an inflammatory disease affecting the uveal layer of the eye. It accounts for about 10–15% of all cases of total blindness in the USA [39]. Diabetic retinopathy is another leading cause of vision-loss globally, affecting adults aged 20–74 years. Of an estimated 285 million people with diabetes mellitus worldwide, approximately one third have signs of diabetic retinopathy [40].

All these pathologies are influenced by a series of risk factors and are characterized by their own way of pathophysiology in AH secretion/ outflow. In most glaucomas, it was established an increased resistance through the trabecular meshwork that contributes to elevated IOP and influence on AH's composition [26]. Diabetes mellitus is associated with problems of general circulation. Ocular effects are dependent on the duration of diabetes, the age of the patient, and the severity of retinopathy. They include changes in AH dynamics, IOP, aqueous flare, permeability of blood-ocular barrier, and retinal vasculature [22, 26]. Uveitis is characterized by inflammation that can cause iris atrophy and secondary glaucoma in some patients. Several studies pointed out different changes in AH due to the increased permeability of the blood-aqueous barrier [5, 26].

The exact number of human AH constitutes is unknown, and it is possible that tens if not hundreds of components exist in the AH and many of these could fall below current detection limits and difficulty in collecting a big quantity for the biochemical exam due to the small eye's chambers. Specific markers for main pathologies represent nowadays an important field of research. Therefore, it was decided to select the most important constitutes from AH for glaucoma, diabetic retinopathy and uveitis, and to analyze their concentration, properties changes.

So, one of the main constitutes of AH is *ascorbic acid* (*Vitamin C* with a concentration about 10- 15 times greater in the AH than in plasma) has the role of antioxidant, protecting the eye from the deleterious effects of free radicals and toxins [10, 21, 41-43]. The concentration of ascorbate is about 15 times greater in the AH than in plasma, suggesting that vitamin C may protect against harmful factors within the eye [10, 41]. It was detected in cornea, AH, lens, vitreous humor, and retina [41, 44].

However, Vitamin C concentrations in AH are lower in patients with various ophthalmic diseases. At the patients with age-related cataract (from 50 to 70 years old) the concentration of vitamin C in AH decreases suggesting that this phenomenon may play a role in susceptibility to cataract formation in older people [44, 45]. Vitamin C concentrations are lower in patients with exfoliation syndrome and glaucoma [46-49]. The endotoxin-induced ocular inflammation in uveitis caused a decrease in the concentration of ascorbic acid in the AH [50]. Diabetic patients have an imbalance between free radical generation and antioxidant defense (vitamin C, vitamin E) which may play a role in the progression of diabetic retinopathy [51].

The low concentration of *proteins* in AH (0,02g at 100ml comparative to plasma concentration of 7g at 100ml) is essential for maintaining the optical transparency [52], this is due to the blood-aqueous barrier. AH comprises many proteins with various roles and important biological functions. The exact number and concentration of human AH proteins are unknown, as it is supposed that tens if not hundreds of lower abundance proteins exist in the AH and many of these could fall below current detection limits [37]. Most of the proteins identified had catalytic, enzymatic, and structural properties [33]. The most abundant proteins found in normal AH are albumin, immunoglobulin G (IgG), transferrin, haptoglobin and antitrypsin that represent the major ones [32, 33]. In a healthy eye, IgG is present at a concentration of approximately 3mg/100ml, while IgM, IgD, IgA are absent due to their large molecule structure [53].

Amount of proteins and cells in AH was observed after surgery, paracentesis, or uveitis [54]. In Prata T. et al.'s study it was mentioned that the total protein concentration in primary open-angle glaucoma AH was approximately two times higher than that in non-glaucomatous patients, albumin (50% of all the protein content) and transferrin being the most abundant protein [55, 56]. Although, it is considered that the alterations in the protein composition of AH trigger signaling molecules that modify the trabecular meshwork and increasing resistance to outflow and induce glaucoma [57]. Grus E.H. et al. found that transthyretin was one of the proteins that are highly abundant in the aqueous of glaucoma patients. It might play a role in the onset of glaucoma since it has been shown to form amyloid deposits (increasing intraocular pressure by the particles that could cause outflow obstructions) [58].

The pathogenesis of uveitis is associated with abnormal expression of some proteins and aberrant regulation of multiple signaling pathways [59]. The blood-aqueous barrier breaks down [60] and the composition and concentrations of proteins in aqueous are similar to that of plasma [61]. The concentration of IgG increases and IgM and IgA appear [62-64]. When the AH proteins concentration rises significantly above its normal level approximately 20mg/100ml, the resultant light scattering (Tyndall effect) makes visible at slit-lamp [5]. Other sources of proteins are represented by IL-1β, IL-2, IL-6, and IL-10, which are cytokines that actively participate in the pathogenesis of clinical uveitis, and it is higher in the samples of patients with uveitis [65, 66].

In diabetic patients, the proteome composition of AH suffers change too [67, 68]. Chiang S.Y. et al. identified 11 proteins differentially expressed between diabetic retinopathy and control groups. There were detected at lower levels – SERPINF1 (encoded protein is secreted and strongly inhibits angiogenesis) and prostaglandin-H2 D-isomerase (PTGDS – involved in development and maintenance of the blood-retina, blood-aqueous humor barrier) compared to control [68, 69]. These altered proteins are involved in in-

flammation, lipid metabolism and cell proliferation, microstructure reorganization, angiogenesis, anti-oxidation, and neuroprotection [67, 69, 70].

Other important protein found in diabetic AH that has an important role in angiogenesis is vascular endothelial growth factor (VEGF) [71, 72]. Data from several studies support the generally accepted supposition that the VEGF level in the aqueous liquid collected from the anterior chamber adequately reflects the VEGF activity in retinal tissues [72, 73]. The severity of retinopathy and the degree of retinal ischemia is directly proportional to the elevation of VEGF levels (957 pg/ml as detected in Patel J.I.'s study) [72, 74-76].

Glucose levels in AH correlate with blood glucose levels [77]. It is a component of the AH due to the process of diffusion. At young patients, the concentration of the glucose in AH represents 76% from the plasma concentration, but with the age the concentration decrease is 63% [12]. Davies P.D. et al. have observed mean AH glucose concentration in non-diabetic is 3.2mmol/L (57.6mg/dl). There were determinate differences between non-diabetic and diabetic patients. The glucose levels in non-diabetic patients were 5.8 mM in plasma and 3.2 mM in AH, while the values for diabetics were 14.2 and 7.8 mM [78], influencing the metabolism of the lens, the refraction [12]. In addition, the glucose level influences the IOP (intraocular pressure) in patients with uncontrolled diabetes that was significantly higher [79, 80]. The mechanism is still unclear, but *in vitro* studies suggested that high glucose conditions could induce excess extracellular matrix synthesis by trabecular meshwork cells. Accumulation of extracellular matrix in the trabecular meshwork blocks the aqueous outflow [81, 82]. Glucose levels of AH in ocular inflammations as iritis, keratitis and corneal ulcer are elevated, according to Alaerts et al. [83]. There is no evidence about the concentration of glucose in glaucoma.

Anyway, the changes in the most important constitutes of the AH involve modifications in the other components (ions, amino acids etc.), physiological properties and cause pathological conditions in the anterior segment. All the biochemical researches made on specific marker in the AH for eye pathologies are developing.

Conclusions

This study reveals significant variations in the differentially abundant changes in human aqueous humor that may be relevant for future diseases treatment in order to get favorable outcomes in patients. The aqueous humor proper composition is important in the regulation of the homeostasis of the ocular tissues. Every pathology leads to changes to aqueous humor. They influence physiological properties and cause pathological conditions in the eye. The specific identification of these markers will aid in understanding various eye diseases of the anterior segment such as glaucoma, uveitis and diabetic retinopathy. Other areas for future study include determining differences in aqueous humor constitutes levels among patients in different age groups.

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