INTRODUCTION

Glaucoma is an optic neuropathy in which at least one eye has accelerated ganglion cell death characterized by excavated cupping appearance of the optic nerve with progressive thinning of retinal nerve fiber layer tissue and corresponding subsequent visual field loss. [1] Glaucoma is the second leading cause of preventable blindness worldwide. Despite availability of medical and surgical treatment many patients with glaucoma currently continue to lose vision. [2] So there is need for novel treatment to complete existing therapies. [3]

According to the World Health Organization (WHO) Glaucoma affects more than 70 million people worldwide with approximately 10% being bilaterally blind, 4.5 to 5 million people are blind around the world because of glaucoma.[2] Risk factors for glaucoma include increased pressure in the eye, a family history of the condition, migraines, high blood pressure, and obesity. Clinically, it is well accepted that the major risk factor for glaucoma is elevated intraocular pressure (IOP). [4]

The history of glaucoma pharmacology began nearly 150 years ago but has followed an exponential curve as most of the drugs used routinely to control intraocular pressure IOP have become available in just the last 35 years.[5]

Once the concept of IOP reduction for glaucoma took hold in 1862, the first IOP-lowering medication introduced

which is the calabar bean. The calabar bean is the original source of physostigmine, a potent miotic. Pilocarpine, the second miotic, came along just a year later and was much better tolerated.[6]

The second class was the adrenergic agonists in 1901. Epinephrine did not become commercially available for glaucoma management until the 1950s, followed soon after by clonidine.[6]

Systemic carbonic anhydrase inhibitors emerged in the late 1930s and early 1940s. Topical carbonic anhydrase inhibitors dorzolamide marketed as Trusopt won FDA approval in 1995.[7]

Propranolol was the first beta blocker, introduced in 1967, and was quickly noted to lower IOP after intravenous administration. The drug was not viable as a topical agent because of both corneal anesthetic properties and a negative effect on tear production. Other candidate beta blockers had additional limitations, such as profound dry eyes syndrome, subconjunctival fibrosis, and tachyphylaxis. Timolol was approved by the FDA in 1978 the beginning of the modern age of glaucoma pharmacology. [8]

For the next 20 years, timolol represented the optimal first-line therapy for most patients with glaucoma. Soon the relatively cardio selective beta blocker betaxolol was approved

for use in the United States, in the early 1980s. Betaxolol has a somewhat more favorable safety profile in patients with pulmonary disease, but the drug is generally regarded as less effective in lowering IOP compared with the non-selective beta blockers.[9] Although these drugs have largely been displaced as first line therapy by the prostaglandin analogs, they remain in common use as adjunctive therapy.

Perhaps no class of IOP-lowering drugs has changed the therapeutic landscape as dramatically as the prostaglandin analogs, the safest and most effective glaucoma drugs to date. Because of these two key characteristics, the prostaglandin class of IOP lowering agents quickly supplanted beta blockers as the preferred first-line agents for most patients with glaucoma.[10]

Despite of use many drugs to modify the course of the disease, none of the current medications for POAG is able to reduce the IOP by more than 25%–30%. Also, some glaucoma patients show disease progression despite of the therapeutics.

New drugs are developed rapidly with novel ideas of action mechanisms for treatment of glaucoma. New agents were invented to lower the IOP through induction of metalloproteinases (MMPs), contraction of trabecular meshwork cells, inhibition of aqueous humor secretion, and activation of CB-1 receptor, etc. The second class of drugs under development is intended to improve the ocular blood

flow, particularly in retina and optic nerve head. Neuroprotection is the latest developed mechanisms of glaucoma treatment.[11]

Ripasudil hydrochloride hydrate (K-115), a specific Rho-associated coiled-coil containing protein kinase (ROCK) inhibitor, is ophthalmic solution developed for the treatment of glaucoma and ocular hypertension in Japan. Topical administration of K-115 decreased intraocular pressure (IOP) and increased outflow facility.[12]

Rho kinase (ROCK) inhibitors are a novel potential class of glaucoma therapeutics with multiple compounds currently in Phase II and III US Food and Drug Administration trials in the United States. These selective agents work by relaxing the trabecular meshwork through inhibition of the actin cytoskeleton contractile tone of smooth muscle. This new mechanism aims to increase outflow facility in TM.[13]

Considerable evidence has shown that TM cells are highly contractile and play an active role in aqueous humor dynamics. It has been shown that TM tissues possess smooth muscle cell-like properties. The contraction and relaxation properties of TM cells are regulated by several enzymes, which have become experimental therapeutic targets for lowering IOP. [14] Cellular properties of the trabecular meshwork are critical for conventional outflow.

There are high levels of RhoA in TM cells that induce a contractile morphology, increased actin fibers, increased focal cell to cell adhesions, increased levels of phosphorylated myosin light chain (MLC) and increased extracellular matrix protein production. These changes will decrease aqueous humor drainage because of cellular and morphological changes in the TM cells. [15] The ciliary muscle (CM) also plays an important role in the conventional route. As contraction of the CM leads to increased trabecular meshwork pore size and increased aqueous drainage.

Moreover, a significant number of patients presenting with glaucoma continue to lose vision despite responding well to therapies that lower eye pressure non-IOP-dependent, so enhancement of optic nerve blood supply and neuroprotection are potential treatment strategies for glaucoma. Significantly elevated levels of RhoA have been detected by immunostaining in the optic nerve head of glaucomatous eyes compared with age-matched controls, reinforcing the association of Rho proteins and glaucoma pathophysiology. [16]

Aim of the Essay

Aim of the essay is to study the effect of Ripasudil K-115 as a member of Rho-associated kinase inhibitors (ROCK inhibitors) in treatment of glaucoma.

ANATOMICAL BACKGROUND

The ciliary body is the site of aqueous humour production and it is totally involved in aqueous humour dynamics. The ciliary body is the anterior portion of the uveal tract, which is located between the iris and the choroid. [17].

The ciliary muscle consists of longitudinal, circular and oblique muscle fibers [18]. Contraction of the longitudinal muscle will opens the trabecular meshwork and Schlemm`s canal. [Figure 1] The circular fibers are the anterior and inner portion, and run in circular manner parallel to the limbus. These fibers inserted in the posterior iris surface. Contractions of it relax the zonules, increase the lens axial diameter and convexity. The oblique fibers connect the longitudinal and circular fibers. The contraction of these fibers may widen the uveal trabecular spaces. [18]

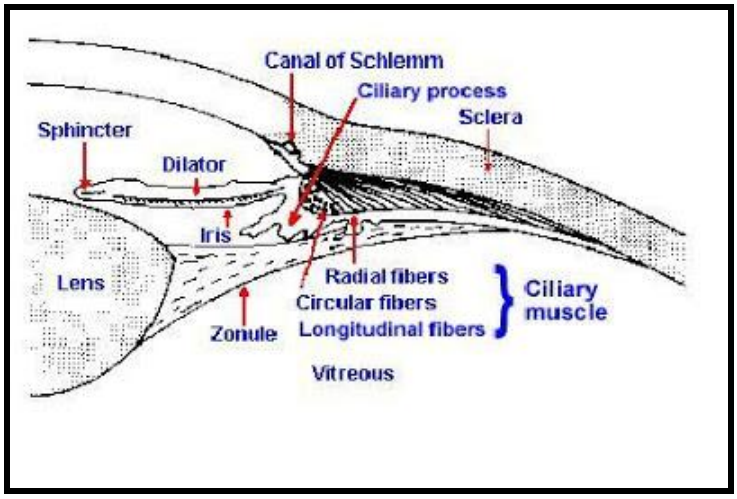

Figure (1): Anatomy of ciliary body, ciliary muscle. [18]

Ciliary process anatomical structure composed of central core of stroma and capillaries covered by a double layer of epithelium[19]. The capillary endothelium is thin and fenestrated. The stroma is very thin, surrounds the vascular tissues and separating them from the epithelial layers.

The stroma is composed of ground substance which is mucopolysaccharides, proteins and plasma of low molecular size, collagen connective tissue and cells of connective tissue and the blood. [20]

The aqueous humor is formed by the ciliary process passes from posterior chamber to the anterior chamber through the pupil, and leaves the eye through the anterior chamber angle. Most of the aqueous humor leaves the eye through the trabecular meshwork, which is called the conventional or canalicular pathway, and accounts for 85 to 95% of aqueous humor outflow of normal human eyes. The other 5-15% of the aqueous humor leaves the eye through the uveoscleral (unconventional pathway) including anterior ciliary muscle and iris to reach supra ciliary and supra choroidal spaces [17].

PHYSIOLOGY OF AQUEOUS SECRETION AND DRAINAGE

Physiology of aqueous humor secretion

Aqueous humor

AH is a transparent fluid present in the anterior and posterior chambers of the eye and is formed by the ciliary epithelium (CE) of the ciliary processes projecting from the CB. AH is formed by selective transfer of solutes (ions, glucose, ascorbate, amino acids and other solutes) and water from the blood across the CE. The fluid is continuously secreted by the CE and enters first into the posterior chamber. It then seeps forward through the narrow space between the lens and the iris and enters the anterior chamber through the pupil. From the anterior chamber it leaves the eye mostly by bulk flow (a pressure dependent flow) through the anterior chamber angle.[21]

Functions of aqueous humor

AH is a nutritive fluid that serves as a blood substitute for the avascular cornea, lens, anterior vitreous and also the trabecular meshwork (TM) of the outflow pathway. AH supplies nutrients and oxygen to these avascular tissues through diffusion. It also removes metabolic wastes of the avascular tissues through its continuous formation, and passage through the ocular chambers and drainage from the eye to the venous blood. Hydrostatic pressure due to AH form the IOP, which

inflates the eye to maintain proper alignment of the optical structures. AH also serves to transport ascorbate, an antioxidant agent in the anterior segment. Presence of immunoglobulins in the AH indicates a role in immune response to defend against invading pathogens.[21]

Histological features of the ciliary processes

The ciliary processes form from connective stroma core contains a mass of capillaries, with the ciliary epithelium CE covering the ciliary processes. The CE consists of an inner layer of non-pigmented epithelium (NPE) and an outer layer of pigmented epithelium (PE). The endothelium of the ciliary capillaries is highly fenestrated so a blood ultra-filtrate fills the stroma. This contains almost all components of the plasma except the blood cells. It is now generally believed that AH is formed mostly by active transport of ions and solutes across the CE. Selective transport of solutes takes place from the stromal fluid across the bilayer into the posterior chamber and this causes a subsequent osmotic flux of water, producing the AH. The contemporary view is that ions and other solutes driven inward from the blood side by the PE cells readily pass through the gap junctions into the NPE cells. [22]

From the NPE, ions and solutes are then secreted across the basolateral membrane into the posterior chamber. [23]

Special features of the CE

The bilayer CE consists of the columnar non-pigmented (NPE) and the cuboidal pigmented epithelial cells (PE). The basal surface of the NPE cells lines the posterior chamber whereas the basal surface of the PE cells rests on the ciliary body stroma (Figure 2). The apices of the PE and NPE cells are in contact with each other and are connected via gap junctions. Despite this arrangement, the secretory process is directed from apex to the base of the NPE cells along the lateral intercellular canals, which are 'closed' at the apical ends by dense junctional complexes, the tight junctions. The coordinated function of the two epithelial cell layers is of importance, since the secreted aqueous must be derived from the blood contained in the capillaries of the ciliary stroma and secretion must occur across both layers.[24]

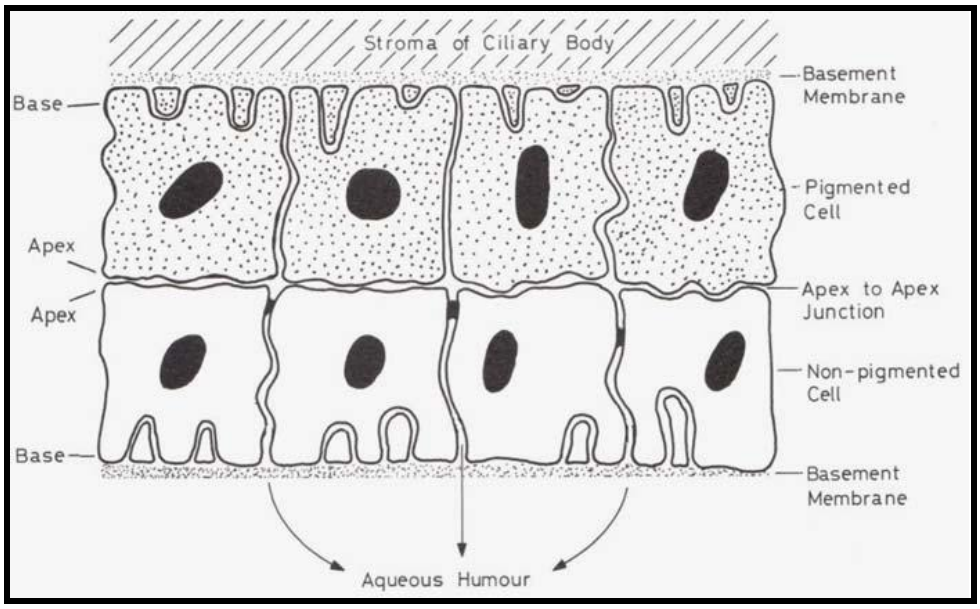

Figure (2): Schematic diagram of ciliary epithelium bilayer showing apex to apex arrangement of pigmented and non-pigmented ciliary epithelium [24]

Aqueous Humor Formation and composition

Three mechanisms are involved in aqueous humor formation: diffusion, ultrafiltration and active secretion. Diffusion occurs when solutes, especially lipid soluble substances, are transported through the lipid portions of the membrane of the tissues between the capillaries and the posterior chamber, proportional to a concentration gradient across the membrane. [23]

Ultrafiltration is the flow of water and water-soluble substances, limited by size and charge, across fenestrated ciliary capillary endothelia into the ciliary stroma, in response to an osmotic gradient or hydrostatic pressure.[23]Diffusion and ultrafiltration are responsible for the accumulation of plasma

ultrafitrate in the stroma, behind tight junctions of the non-pigmented epithelium, from which the posterior chamber aqueous humor is derived.[25]

Active secretion is thought to be the major contributor to aqueous formation, responsible for approximately 80% to 90% of the total aqueous humor formation. [26]

The main site for active transport is believed to be the non-pigmented epithelial cells. Active transport takes place through selective trans-cellular movement of anions, cations, and other molecules across a concentration gradient in blood-aqueous barrier. This is mediated by protein transporters distributed in the cellular membrane. The energy required for the transport is generated by hydrolysis of adenosine triphosphate (ATP) to adenosine diphosphate (ADP), which is activated by Na^+ and K^+ mediated by Na^+-K^+-ATPase, an enzyme located in both the non-pigmented and pigmented ciliary epithelia.[27]

Another enzyme, carbonic anhydrase, found in the non-pigmented ciliary epithelia, mediates the transport of bicarbonate across the ciliary epithelium by the reversible hydration of CO_2 to form HCO_3^- and protons. Bicarbonate formation influences fluid transport by affecting Na^+, possibly by regulating the pH for optimal active ion transport.[28] The movement of electrolytes across the ciliary epithelium is regulated by electrochemical gradients and, although there is a

net direction of secretion across the epithelium, hydrostatic and oncotic forces favor resorption of aqueous humor.[29] Chloride ion is the major anion transported across the epithelium through Cl^- channels. Other molecules are also actively transported, including ascorbic acid, which is secreted against a concentration gradient by sodium-dependent vitamin C transporter [30] and certain amino acids, which are secreted by at least three different solute carriers. Active transport produces an osmotic gradient across the ciliary epithelium, which promotes the movement of other plasma constituents by ultrafiltration and diffusion. [31]

The rate of aqueous humor turnover is estimated to be 1.0% to 1.5% of the anterior chamber volume per minute, which is 2.4 ± 0.6µl/min.[32] Using fluorophotometry, diurnal variations were observed in aqueous humor turnover rates, reflecting a pattern known as the circadian rhythm of aqueous humor flow in humans. Aqueous humor flow is higher in the morning than at night. Aqueous humor flow is normally about 3.0µl/min in the morning, 2.4µl/min in the afternoon, and drops to 1.5µl/min at night.[32] The mechanism that controls this biologic rhythm is poorly understood. Circulating epinephrine available to the ciliary epithelia may be a major driving force. [33]

Aqueous humor composition depends not only on the nature of its production, but also on the metabolic interchanges that occur within various tissues throughout its intraocular route. The major components of the aqueous humor are organic

and inorganic ions, carbohydrates, glutathione, urea, amino acids and proteins, oxygen, carbon dioxide and water. Aqueous humor is slightly hypertonic to plasma, [34]

The greatest differences in aqueous humor relative to plasma, are the concentrations of protein (200 times less) and ascorbate (20 to 50 times higher).The protein content of aqueous humor has both quantitative and qualitative differences compared to plasma. Most aqueous humor proteins are intrinsic glycoproteins of the vitreous, which are secretory products of the inner epithelial layer of the ciliary body.[35]Specific classes of immunoglobulins, such as IgG, were found to be in a higher concentration in the aqueous humor as compared to IgM and IgA levels.[36] Relative concentrations of free amino acids vary, with ratios to plasma concentration ranging from 0.08 to 3.14, reinforcing the concept of active transport of amino acids.[37]

Glucose and urea in the aqueous humor are approximately 80% of the plasma levels. Important anti-oxidant substances can also be found in the aqueous humor, such as glutathione and ascorbate. A number of molecules involved in the maintenance of the extracellular matrix, such as collagenase, have been identified in human aqueous humor, which may influence TM outflow resistance and, consequently, the IOP.[37]